BACKGROUND
Interleukin-2 is a Promising Cytokine For Immunotherapy
Cytokine therapy could become a pillar of cancer immunotherapy given its potential to activate the immune system and promote antitumor activity. However, many cytokine therapies are limited in the clinic due to dose limiting toxicities associated with their administration. Recent approaches to improve upon IL-2 therapy have focused on developing attenuated forms of the cytokine (Non-Alpha forms) that are unable to bind to the high affinity IL-2 receptor, aiming to reduce the off-target toxicity and to minimize potential Th suppressive effects. However, Non-Alpha forms of IL-2 may also have limited ability to activate the tumor specific effector cells that drive antitumor immune responses. Indeed, modeling suggests that Non-Alpha molecules will need to be dosed at >100 times the amount of a therapeutic delivering wild type IL-2 to generate similar receptor occupancy in the tumor microenvironment. To address the shortcomings of 0.2-0.4 mg/kg we have developed WTX-124, a conditionally activated INDUKINE™ molecule that is designed to take advantage of the deregulated protease milieu in the TME to deliver native IL-2 in a targeted fashion to tumor tissues after systemic administration. WTX-124 is better tolerated than half-life extended IL-2 in mice and generates robust anti-tumor immunity in several pre-clinical models. To better understand the potential of IL-2 therapeutics targeting various proteases or Non-Alpha IL-2 as a payload, we created an IL-2 INDUKINE molecules containing a Non-Alpha IL-2 moiety as reported in recent publications [1-2] and compared it in its vitro and in vivo activity of that of WTX-124.

CONCLUSIONS and REFERENCES
- WTX-124 is substantially more active than a Non-Alpha IL-2 variant when tested on activated T cells, due to induction of high affinity receptor (CD25/CD122/CD152) for IL-2.
- WTX-124, an INDUKINE molecule containing wild type IL-2, generated robust antitumor activity in the MC38 model and promoted the required and evasion of tumor specific CD8+ T cells.
- Meanwhile, a variant INDUKINE molecule containing Non-Alpha IL-2 failed to generate antitumor activity, to drive tumor specific CD8+ T cell expansion, or to activate tumor infiltrating immune cells when dosed up to 100 higher than therapeutic doses of WTX-124.
- While both INDUKINE molecules protected tumor infiltrating CD8+ T cells from extinction, only WTX-124 was able to induce an effector phenotype in tumor specific CD8+ T cells.
- Only treatment with WTX-124 resulted in clustering of CD8+ T cells with CD103+ cells present downstream.

IL-2 Protects CD8+ T Cells from Exhaustion
WTX-124 Strongly Protects Tumor Specific T Cells
MCB8 tumor bearing mice were dosed as previously described. Doses are specified in the figure. The tumors were harvested on Day 5 or Day 11 for analysis. Tumors were dissociated and analyzed by flow cytometry after ex vivo restimulation for cytokine staining at the specified timepoints. A) Representative flow cytometry plots and B) quantification. Statistics were generated using a two-way ANOVA, and significance is reported as follows: ** = p<0.01; *** = p<0.001.

WTX-124 Drives Clustering of CD8+ T Cells with CD103+ DCs
Suggestive of Ongoing T cell Activation Within the TME
MCB8 tumor bearing mice were dosed as previously described. Doses are specified in the figure. The tumors were harvested on Day 5 for analysis. Multiple immunoassay analysis was performed on IFN-γ positive cells, with the various markers examined specified in the figure.